Design, Synthesis, and Biological Evaluation of the First c-Met/HDAC Inhibitors Based on Pyridazinone Derivatives

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S Supporting Information

ABSTRACT: Simultaneous blockade of more than one pathway is considered to be a promising approach to overcome the low efficacy and acquired resistance of cancer therapies. Thus, a novel series of c-Met/HDAC bifunctional inhibitors was designed and synthesized by merging pharmacophores of c-Met and HDAC inhibitors. The most potent compound, **2m**, inhibited c-Met kinase and HDAC1, with IC_{50} values of 0.71 and 38 nM, respectively, and showed efficient antiproliferative activities against both EBC-1 and HCT-116 cells with greater potency than the reference drug Chidamide. Western blot analysis revealed that compound **2m** inhibited phosphorylation of c-Met and c-Met downstream signaling proteins and increased expression of Ac-H3 and p21 in EBC-1 cells in a dose-dependent manner. Our study presents novel compounds for the further exploration of dual c-Met/HDAC pathway inhibition achieved with a single molecule.

KEYWORDS: Dual c-Met/HDAC inhibitor, hybrid, designed multiple ligand (DML)

c-Met, which is encoded by the Met proto-oncogene, is a receptor tyrosine kinase (RTK) whose binding to hepatocyte growth factor (HGF) leads to the recruitment of a number of adaptor proteins or effectors, such as PI3K-AKT-mTOR and Ras-Raf-MEK-ERK, through downstream signaling pathways that mediate cellular properties, including proliferation, survival, migration, mitogenesis, morphogenesis, and angiogenesis.¹ Aberrant c-Met activation, mutation, amplification, and translocation play important roles in cancer formation, progression, dissemination, and drug resistance.² Moreover, increased levels of both c-Met and HGF are associated with poor clinical outcomes in cancer patients.³ Thus, c-Met kinase has received considerable attention as an attractive target for cancer treatment. However, similar to other RTK inhibitors, due to complex factors, such as network robustness, bypass crosstalk, and compensatory activities,⁴ c-Met inhibition alone is usually not sufficient to block tumor progression, exhibiting low efficacy or acquired resistance in clinical trials.

Human histone deacetylases (HDACs) are a family of 18 enzymes that function by deacetylating histone and nonhistone proteins and can be divided into four classes.^{5,6} In view of their fundamental roles in gene expression, HDACs have been

associated with basic cellular events and disease states, including cell growth, differentiation, and cancer formation.^{7,8} Moreover, overexpression of HDACs has been found in a variety of human cancers.⁹ Therefore, HDAC inhibitors (HDACi) have emerged as promising new therapeutic agents for treating cancer. Thus far, five HDACi have been launched on the market (Figure 1).^{10–12}

HDACs influence c-Met and its downstream signaling pathways both directly and indirectly. HDACi inhibit HGF production.¹³ In addition to histones, HDACi disrupt the deacetylation of nonhistone proteins, including key tumor suppressors and oncogenes, such as tubulin, p53, Hsp90, and Bcl-2, which can lead to the degradation of pro-growth/pro-survival client proteins (e.g., Raf, AKT, c-Src, etc.).^{14,15} Additionally, recent evidence has suggested that HDACi exert antiproliferative effects against c-Met-dependent cells.^{16,17}

Received: April 22, 2017

Accepted: July 18, 2017

Published: July 18, 2017

Figure 1. Structures of representative HDACi.

There are two main approaches to overcome the low efficacy of and acquired resistance to RTK inhibitors. Compared to combination therapies, designed multiple ligand (DML), a single molecule with multiple targets, avoids drug–drug interactions, off-target adverse effects, poor patient compliance, and high development costs.¹⁸ Considering that HDACi synergize with RTK inhibitors,^{19,20} modulation of RTK pathways by inhibition of HDACs has attracted increasing interest in cancer chemotherapy. Significant progress in combining the HDAC pharmacophore with kinase inhibitory activities was recently described (e.g., CUDC-101 and CUDC-907) (Figure 2).^{21–26}

Figure 2. Structures of representative dual RTK/HDAC inhibitors.

Given the aforementioned evidence, we hypothesized that the development of a single molecule that concurrently inhibits HDAC and c-Met activities would serve as a promising strategy for related cancer treatments. In this study, we designed the first c-Met/HDAC bifunctional inhibitors by hybridizing the pharmacophores of HDACi with a c-Met inhibitor. The rationale for the DML design originated from our previous structure–activity relationship (SAR) study and modeling work, which showed that the pyridazinone-quinazoline moiety of the selective c-Met inhibitor 1 occupies the hinge region of the ATP binding site to form two hydrogen bonds with the residues of c-Met protein, and the substituent at C7 of the quinoline moiety extends into the solvent-exposed region of the protein.²⁷ The structure–activity relationship (SAR) results revealed that the modification of side chains had no significant effect on c-Met inhibitory activity. For the classic pharmacophoric model of HDACi,²⁸ the zinc binding group (ZBG) of HDACi plays an essential role in HDAC inhibitory activity. Meanwhile, the surface recognition cap group (CAP) accommodates the pyridazinone-quinazoline moiety of compound 1 to interact with the rim of the catalytic tunnel of HDAC enzymes. Therefore, we incorporated a ZBG at the C7 position of the quinoline moiety of 1 with a proper linker to design a new DML (Figure 3). Herein, we report the synthesis

and biological activities of these hybrid c-Met/HDAC inhibitors.

Figure 3. Design strategy of dual c-Met/HDAC inhibitors.

Results and Discussion. Chemistry. The route employed to synthesize target compounds 2a–2n is outlined in Scheme 1.

Scheme 1. Synthesis of Compounds 2a–2n^a

^aReagents and conditions: (a) (i) glyoxylic acid monohydrate, 120 °C, (ii) hydrazine hydrate, 100 °C, 25%; (b) 2-(*tert*-butoxy)ethanol, NaH, DMF, 40 °C, overnight, 65–88%; (c) TFA, rt, 30–60 min, 90–97%; (d) (i) MsCl, Et₃N, THF, 0 °C, 30 min; (ii) 3, Cs₂CO₃, DMF, 50 °C, 5 h, 60–85%; (e) 4 N BBr₃, anhydrous CH₂Cl₂, 0 °C, rt, 5 h, 57%; (f) or Cs₂CO₃, DMF, 50 °C, 5 h, 33–91%; (g) Et₃SiH, TFA, CH₂Cl₂, rt, 1 h, 34%; (h) 50% NH₂OH in H₂O, MeONa, MeOH, rt, overnight, 44–67%; (i) LiOH·H₂O, THF/H₂O, rt, overnight, 57–95%; (j) amines, PyBOP, Et₃N, DMF, rt, overnight, 20–45%.

The pyridazinone intermediate 3 was synthesized from acetylpyrazole with glyoxylic acid and hydrazine. Simultaneously, 4-chloro-7-substituted quinolines were reacted with 2-(*tert*-butoxy)ethanol to produce the corresponding *o*-ethoxy derivative 4, which was transformed to the alcohol 5. After mesylation of the hydroxyl group in 5, the pyridazinone 3 was transformed to the pyridazinone-quinolones 6a–6b and 9n by *N*-alkylation. Compound 6a was converted to 7, which was followed by alkylation with the corresponding bromides to afford 8a–8n and 2g. Next, hydroxamic acids 2a–2d were obtained from 8a–8d by reaction with NH₂OH in MeOH. The trityl group of 8f was removed with trifluoroacetic acid and triethylsilane to afford 2f. Alternatively, 8e and 8h–8n were converted to the corresponding carboxylic acids (9) followed by condensation with the corresponding amines to yield compounds 2e and 2h–2n.

After Suzuki or Buchwald cross-coupling reactions,^{24,29} **6b** (Scheme 2), **10a**, and **10b** were obtained. Compound **10c** was

Scheme 2. Synthesis of Compounds **2o–2p**^a

^aReagents and conditions: (a) 5-boronothiophene-2-carboxylic acid, Pd(dppf)₂Cl₂, Cs₂CO₃, 1,4-dioxane, 100 °C, overnight, 87%; (b) methyl 5-aminopicolinate, Pd(OAc)₂, S-Phos, Cs₂CO₃, toluene, 110 °C, overnight, 31%; (c) LiOH·H₂O, THF/H₂O, rt, overnight, 85%; (d) *o*-phenylenediamine, PyBOP, Et₃N, DMF, rt, overnight, 57% and 37%.

generated from **10b** by hydrolysis. Finally, the carboxylic acids **10a** and **10c** were coupled with benzene-1,2-diamine to afford the target compounds **2o** and **2p**, respectively.

Biological Results. c-Met and HDAC1 enzymatic assays, as well as EBC-1 cell (c-Met addicted) and HCT116 cell assays were conducted to evaluate the final compounds. The results are summarized in Tables 1–3.

Table 1. SAR Study on the Chain Length of Hydroxamic Acids **2a–2d**

compd	<i>n</i>	Enzymatic activity		Cellular activity	
		c-Met (IC ₅₀ nM)	HDAC1 (IC ₅₀ nM)	EBC-1 (IC ₅₀ μM)	HCT-116 (IC ₅₀ μM)
2a	2	1.0 ± 0.0	>1000	0.87 ± 0.08	>10
2b	3	0.96 ± 0.20	>1000	0.37 ± 0.05	>10
2c	4	0.73 ± 0.06	216 ± 24	0.46 ± 0.33	>10
2d	5	0.54 ± 0.04	18 ± 7	0.21 ± 0.01	>10
1		0.84 ± 0.06	>1000	0.060 ± 0.010	>10
SAHA		N.T. ^a	44 ± 4	1.3 ± 0.1	1.4 ± 0.6

^aNot tested.

Considering the prevalence of the hydroxamic acid group in HDACi and its strong chelation ability with zinc ion,^{21–26} we first introduced hydroxamic acid as a ZBG to the side chain based on the c-Met inhibitor **1**. Compounds **2a–2d** displayed excellent c-Met kinase inhibitory activities, with IC₅₀ values in the low nanomolar range, similar to compound **1** (Table 1). These results suggest that the c-Met kinase inhibition exhibited by the compounds was unaffected by changing the carbon length of the side chain. For HDAC1 inhibition, analogues **2a** and **2b**, with short side chains, did not exhibit apparent inhibitory activities. Increasing the linker length (six carbons, compound **2d**) enhanced HDAC1 inhibitory activity. However, **2c** and **2d** did not show potency against HCT116 cells.

We also introduced other reported ZBGs, including benzamide, thiols, ketones, and methylamide, at the side chain.^{5,30} As shown in Table 2, the compounds with various ZBGs exhibited excellent c-Met kinase activities and potent antiproliferative activities against EBC-1 cells, which verified the

Table 2. Effects of the ZBG on Biological Activity

Compd	R	Enzymatic activity		Cellular activity	
		c-Met (IC ₅₀ nM)	HDAC1 (IC ₅₀ nM)	EBC-1 (IC ₅₀ μM)	HCT-116 (IC ₅₀ μM)
2e		0.96±0.20	221±37	0.37±0.05	7.0±1.6
2f	SH	1.5±0.1	329±64	0.64±0.20	>10
2g		1.0±0.0	>1000	0.025±0.001	>10
2h		1.2±0.2	>1000	0.079±0.004	>10
1		0.84±0.06	>1000	0.060±0.010	>10
SAHA		N.T. ^a	44±4	1.3±0.1	1.4±0.6

^aNot tested.

flexibility of the side chain in c-Met inhibitory activity. Compared to **2c** and **2d**, compounds **2e** and **2f** showed moderate inhibition against HDAC1. Interestingly, only compound **2e**, with benzamide as the ZBG, possessed antiproliferative activity against HCT-116 cells, with an IC₅₀ value of 7.0 ± 1.6 μM. Conversely, compounds **2g–2h**, with a ketone and a methylamide as the ZBG, respectively, were inactive against HDAC1 and HCT-116 cells at the highest concentrations tested. Thus, benzamide was considered the optimal ZBG for the designed dual-action inhibitors.

Given the importance of the ZBG on dual inhibitory activity, a SAR study and structural modifications were performed, as shown in Table 3. Varying the linker did not affect c-Met inhibition significantly (compounds **2i–2p**, except for **2n**), while the potency of HDAC1 inhibition and antiproliferative activities of EBC-1 and HCT-116 cells were highly influenced. Compound **2i**, with a benzyloxy linker, displayed moderate HDAC1 inhibitory activity, with an IC₅₀ value of 156 ± 24 nM and potent antiproliferative activity against both EBC-1 and HCT-116 cells. However, introduction of cinnamyl or phenethyl to compounds **2j** and **2l** resulted in a loss of their HDAC1 inhibitory activity in HCT-116 cells, and the cellular activities of EBC-1 cells decreased accordingly. In contrast, compound **2n**, which does not contain a linker, lost both its enzymatic and cellular activities. Compounds **2o** and **2p**, with thienyl and aminopyridyl as the linker, respectively, possessed moderate potency for HDAC1 inhibition, with IC₅₀ values of 467 ± 38 and 129 ± 10 nM. However, their potency in HCT-116 cells was not detectable at the highest concentration tested. It is important to note that compound **2m**, with an alkyloxy pyridine linker, displayed the most potent inhibition of HDAC1, with an IC₅₀ value of 38 ± 1.7 nM (4-fold improvement compared to the reference drug Chidamide). Moreover, it also showed good potent inhibitory activity against both EBC-1 and HCT-116 cells.

Western Blot Analysis. Encouraged by the potent inhibitory activity of compound **2m** against both c-Met and HDAC1, Western blot assays were conducted to further confirm the dual-action targeting of c-Met and HDAC1 compared to the c-Met inhibitor **1** and the HDAC inhibitor Chidamide as positive

Table 3. SAR Study on the Linkers of Benzamides 2i–2p

Compd	Linker	Enzymatic activity		Cellular activity	
		c-Met (IC ₅₀ nM)	HDAC1 (IC ₅₀ nM)	EBC-1 (IC ₅₀ μM)	HCT-116 (IC ₅₀ μM)
2i		0.44±0.09	156±24	0.042±0.01	1.8±0.6
2j		1.1±0.1	>1000	0.49±0.10	>10
2k		0.63±0.02	625±122	0.046±0.01	7.9±0.3
2l		5.1±0.7	>1000	0.21±0.01	>10
2m		0.71±0.04	38±2	0.058±0.05	1.3±0.4
2n	--	37±2	>1000	>10	>10
2o		1.3±0.2	467±38	0.12±0.00	>10
2p		3.7±1.0	129±10	0.45±0.28	>10
Chidamide		N.T. ^a	112±20	2.9±0.0	7.8±0.5

^aNot tested.

controls, respectively. The results in Figure 4 show that compound **2m** decreased phosphorylation of c-Met in EBC-1 cells and inhibited phosphorylation of key downstream molecules Akt and Erk1/2 in a dose-dependent manner. These data suggest that **2m** inhibited c-Met activity as well as subsequent c-Met downstream signaling. In addition, the levels of both Ac-H3 and the tumor suppressor gene p21 were

Figure 4. Compound **2m** effectively blocked dual signaling pathways in EBC-1 cells. EBC-1 cells were incubated with compound **2m** at different concentrations for 24 h. Protein expression was detected by immunoblot analysis with a specific antibody.

significantly upregulated by compound **2m** compared to the vehicle control group. However, expression of Ac-tubulin was not affected following treatment with compound **2m**, indicating that **2m** selectively blocks class I HDAC signaling and does not inhibit HDAC6 at the cellular level.

In conclusion, the first dual c-Met/HDAC inhibitors were successfully designed, synthesized, and evaluated by merging the pharmacophores of a c-Met inhibitor and HDAC inhibitor. Following a detailed SAR evaluation, compound **2m**, with nanomolar potency against c-Met and HDAC1 as well as potent antiproliferative activity in both EBC-1 and HCT-116 cells, was found. Western blot analysis showed that compound **2m** inhibited phosphorylation of c-Met and subsequent c-Met downstream signaling and increased expression of Ac-H3 and p21 in EBC-1 cells in a dose-dependent manner. These results indicate that the simultaneous blockade of the c-Met and HDAC pathways represents a promising approach for cancer treatment and may provide useful compounds for further studies of multiple pathway inhibition achieved with a single molecule.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsmmedchemlett.7b00172.

Synthetic procedures, characterization data, and biological assay procedures (PDF)

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This study was supported by grants from the National Natural Science Foundation of China (81225022, 81321092, 81673472, and 81402966) and by the “Personalized Medicines—Molecular Signature-based Drug Discovery and Development”, Strategic Priority Research Program of the Chinese Academy of Sciences (grant no. XDA12020105).

■ ABBREVIATIONS

SAHA, Vorinostat; HDACi, HDAC inhibitor; DML, designed multiple ligand; c-Met, c-Met kinase; HGF, hepatocyte growth factor; HDAC, histone deacetylase; RTK, receptor tyrosine kinase; PI3K, phosphatidylinositol 3-kinase; Bcl-2, B-cell lymphoma-2; c-Src, c-Src kinase; NH₂OH, hydroxylamine; MeOH, methanol; LiOH, lithium hydroxide

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